1: Biochem Biophys Res Commun 1998 Jul 20;248(2):293-6

A sensitive, continuously recording fluorogenic assay for calpain.

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We have developed a sensitive and continuously recording fluorogenic assay for the thiol protease calpain. This assay uses the dipeptide substrate Suc-Leu-Tyr-4-Methoxy-2-Naphthylamine (Suc-LY-MNA) in Tris buffer (pH 7.5) in the presence of 0.1% CHAPS. The assay is linear over a wide range of enzyme concentration and is capable of detecting 10 picomolar calpain making it more sensitive than any previously published method. Moreover, this assay gives a rate that is linear for over ten minutes making it useful for mechanistic studies of inhibitors. This assay can be easily adapted to a 96-well plate format facilitating the large scale screening of inhibitors. Copyright 1998 Academic Press.

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1: Blood 1990 Mar 15;75(6):1273-81

Membrane expression of platelet calpain.

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Platelet calpain has many platelet substrates, including external membrane proteins. We thus investigated whether platelet calpain II was associated with platelet membranes in unstimulated and thrombin-activated platelets. A monospecific, goat polyclonal antibody was reared to purified platelet calpain II. Sixteen whole platelet lysates were found to contain 4.5 +/- 0.7 micrograms calpain antigen II per 10(8) platelets (mean +/- SEM) as determined by a competitive enzyme-linked immunosorbent assay. Using the dipeptide fluorogenic substrate, Suc-Leu-Tyr-MCA, 17 human platelet lysates contained 3.6 +/- 0.4 micrograms calpain activity per 10(8) platelets. Platelet calpain II was associated with the Triton X-100 insoluble platelet cytoskeletons from both unstimulated and thrombin-activated platelets. When compared with the total cell content of platelet calpain II, calpain antigen (10% to 13%) and calpain activity (24% to 28%) was associated with platelet cytoskeletons in unstimulated and thrombin-activated platelets, respectively. On immunoblot, the heavy chain (80 Kd) of calpain II was detected in platelet cytoskeletons. Subcellular fractionation studies on both unstimulated and thrombin-activated platelets, revealed that half of the total platelet calpain II antigen was associated with cytosol, and the other half was associated with the membrane fraction. Platelet calpain II was not seen on the surface of unstimulated, paraformaldehyde fixed platelets by immunofluorescence. However, on thrombin-activated platelets, rim immunofluorescence was seen, indicating that activated platelets externalize their calpain. This observation was confirmed by the finding that about 2,000 molecules per platelet of an 125I-anti-calpain II Fab' specifically bound to thrombin-activated but not unstimulated platelets. Both dibucaine (1 mmol/L) and platelet activating factor (1.86 mumol/L) in the absence of external Ca++, but not collagen (5 micrograms/mL) or ionophore A23187 (2.5 mumol/L) in the absence of external Ca++, were also able to externalize platelet calpain II antigen, as indicated by a similar level of specific 125I-anti-calpain II Fab'-platelet binding. These combined studies indicate that platelet calpain II is a major protein, comprising 2% of total platelet protein, a

substantial portion of which is membrane-associated. When platelets are activated by thrombin and platelet activating factor, calpain II antigen also becomes present on the external platelet surface.

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